

A Preliminary Demonstration of ATR-FTIR Spectroscopy of Red-wattled Lapwing (*Vanellus indicus*) Eggshells for Environmental Chemical Screening, Suggesting Microplastic-Associated Contamination, Indore, India

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Abstract

Simple, non-invasive ways to screen wild birds for the chemical traces of plastic pollution remain scarce. This study tested whether attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy can be applied to bird eggshells as a rapid, non-destructive matrix for environmental chemical screening, and, as a preliminary observation, whether the spectra flag an organic signal worth following up as possible microplastic-associated contamination. Naturally hatched and abandoned eggshells of the ground-nesting Red-wattled Lapwing (*Vanellus indicus*) were collected from two sites in Indore district, central India: Rau, a peri-urban town ($n = 7$), and Jamli, a semi-rural village ($n = 8$), both close to informal garbage dumping. Eggshells were analysed by ATR-FTIR under strict contamination control alongside procedural blanks, and nest soil and settled dust were examined by microscopy. All spectra were dominated by calcite (carbonate bands near 1423 , 874 and 710 cm^{-1} , with overtones near 2516 and 1797 cm^{-1}), confirming an intact mineral framework. A weak aliphatic C–H doublet (~ 2927 and $\sim 2869\text{ cm}^{-1}$) was detected in all 15 shells. Normalised to the carbonate band, the mean C–H ratio did not differ between sites (Mann–Whitney $U = 28$, $p = 1.00$), but its variance was significantly greater at the peri-urban site (Levene $p = 0.005$), suggesting patchier exposure. Blanks were clean, confirming the signal was sample-derived. The 1797 cm^{-1} band is the calcite overtone, not a carbonyl. ATR-FTIR is a feasible eggshell screening tool; polymer confirmation now requires micro-Raman and pyrolysis gas chromatography–mass spectrometry.

Introduction

Plastic pollution is no longer seen as only a marine issue. Over the last decade it has become clear that soils, freshwater, the air and living organisms on land all carry plastic in one form or another (Rillig & Lehmann, 2020; Dris et al., 2016). On land, the plastic comes from many sources at once, such as poorly managed municipal waste, agricultural films, sewage sludge spread on fields, fibres shed from clothes, and the wear of tyres and roads, and it is then moved around by wind and water, including through the air, in cities, towns and villages alike (Jambeck et al., 2015; Mahreen et al., 2025; Prajapati et al., 2025). Weathered plastic can also carry additives and pollutants that stick to its surface, such as polycyclic aromatic hydrocarbons and trace metals, which makes the question of exposure more complicated than counting particles alone (Rochman et al., 2014; Bakir et al., 2013; Gallo et al., 2018).

Birds are useful indicators of this kind of pollution, especially the ones that feed on or close to the ground and are therefore in constant contact with soil and dust (Provencher et al., 2018; Gallo et al., 2018). Plastic particles have been reported from the guts and droppings of many birds, and plastic-associated chemicals have been found in seabirds (Sühling et al., 2022; Fackelmann et al., 2023). What has been studied far less is whether any of this reaches the parts of a bird linked to reproduction, in particular the egg and its shell. It is already well known that mother birds pass metals and persistent organic pollutants into their eggs (Eagles-Smith et al., 2016), and micro- and nanoplastics, or the chemicals in them, have been traced into the eggs and embryos of other egg-laying animals (Pitt et al.,

2018; Chemello et al., 2023; Curl et al., 2024). This makes the eggshell an obvious but under-used place to look.

Eggshells are attractive for this purpose for practical reasons. They are made mostly of calcium carbonate (calcite) held in a matrix of proteins, so they are hard and stable and can hold on to both their own organic material and material picked up from outside (Hincke et al., 2012; Rodríguez-Navarro et al., 2015). They can also be collected without disturbing any living bird, simply by picking up shells left after hatching or from abandoned eggs. ATR-FTIR spectroscopy is a good match for this kind of sample because it is fast, needs almost no preparation and does not destroy the sample, and it is already widely used to look for the functional groups of polymers in environmental samples (Harrison et al., 2012; K  ppler et al., 2018). The catch is that in a biological sample the same infrared bands can come from the material of the shell itself, so the results have to be read carefully and backed up by strict contamination control (Weisser et al., 2021; L  der & Gerdt, 2015).

It is important to be clear about what ATR-FTIR can and cannot do. On its own it cannot prove which polymer is present, or how much, in a mixed biological sample; that needs confirmatory methods such as micro-Raman spectroscopy and pyrolysis gas chromatography–mass spectrometry (pyrolysis-GC/MS) (K  ppler et al., 2018). What it can do well is describe a sample quickly and flag the ones that are worth a closer, more expensive look. As far as we are aware, this screening role has not yet been tried on the eggshells of a wild, ground-nesting bird. The Red-wattled Lapwing (*Vanellus indicus*) is a good species for such a test: it is common across the Indian subcontinent and nests in a simple scrape on open ground, so its eggs sit in direct contact with soil, dust and settled material, and the adults feed on the same surfaces (Arya et al., 2023; BirdLife International, 2024). India is also a fitting place to ask the question, because it is urbanising quickly, uses a great deal of agricultural plastic, and already has reports of plastic in its soils and in settled dust (Mahreen et al., 2025; Prajapati et al., 2025). Against this background, the present study had two aims: first, to show that ATR-FTIR can be applied to lapwing eggshells to describe the shell and detect any organic component without harming a bird; and second, kept deliberately modest, to see whether the spectra flag an organic signal worth following up as possible plastic-related contamination, given that both sites lie close to informal garbage dumping. Throughout, the plastic angle is treated as a suggestion supported by the surroundings, not as spectroscopic proof.

Materials and methods

Study sites

The work was carried out at two sites in Indore district, Madhya Pradesh, central India, chosen because they differ clearly in how built-up and disturbed they are (Fig. 1a and 1b). Rau (22.6364° N, 75.8107° E) is a rapidly urbanising Nagar Parishad lying about 15–17 km south-west of Rajwada, the centre of Indore city. It forms part of the expanding Indore metropolitan region and has mixed residential, commercial, educational and industrial land use, a dense road network and heavy traffic, with an estimated population

of about 113,000 at 550–580 m above mean sea level; near the nesting area, waste disposal is informal, with scattered household garbage typical of a growing town. Jamli (22.5523° N, 75.7606° E) is a village in Dr. Ambedkar Nagar (Mhow) Block, about 24–27 km south-west of Indore city and roughly 7 km from Mhow town; it covers 559.44 ha and, according to the 2011 Census of India, had 767 households and a population of 3,924. The village is mostly farmland and open ground with light traffic, but an informal, uncontrolled garbage-dumping site that acts as a small local landfill lies close to it. Both sites therefore lie within a few kilometres of visible, mixed plastic waste, and this shared exposure to informal dumping is the environmental context for the screening reported here.

Study species and non-invasive collection

The Red-wattled Lapwing lays its eggs in a shallow scrape on bare or sparsely vegetated ground, which keeps the eggs in close contact with soil and settled material (Arya et al., 2023; BirdLife International, 2024). All sampling was non-invasive and opportunistic. Only fragments of eggs that had already hatched or that had clearly been abandoned were collected; no live birds, adults, active nests or viable eggs were touched or disturbed at any point. Seven shells were collected at Rau and eight at Jamli, giving 15 in total (Fig. 2). To avoid adding plastic during collection, the shells were handled with nitrile gloves and stored in pre-cleaned glass containers with aluminium-foil-lined lids, avoiding plastic bags and containers throughout; field staff wore cotton clothing and avoided synthetic fabrics to limit stray fibres (Löder & Gerdt, 2015).

Soil and dust

To describe the local pollution, nest-associated surface soil and settled dust were also collected and examined under a stereomicroscope, the soil after density separation. Suspected microplastics were noted from their shape (fibres, films, fragments) and appearance, including rubber-like tyre-wear particles. Microscopy identifies only *suspected* microplastics and cannot confirm the polymer; no FTIR or other polymer-specific test was run on these samples. They are used only as background context and were never matched, band for band, to the eggshell spectra.

Contamination control and procedural blanks

Samples were processed under low air movement, on surfaces cleaned with ethanol and rinsed with ultrapure water, while wearing cotton coats and avoiding synthetic materials. Procedural blanks were carried through the same steps as the shells and measured in the same way, and were checked before any shell spectrum was interpreted (Löder & Gerdt, 2015; K  ppler et al., 2018). The blank was effectively clean across the diagnostic regions, staying at or near the instrument noise level: 0.003 absorbance units (AU) at 2927 cm⁻¹, 0.002 AU at 2869 cm⁻¹, 0.004 AU at 1423 cm⁻¹, and 0.002 AU or less at 1797, 874 and 710 cm⁻¹. These values are one to two orders of magnitude below the matching bands on the shells, confirming that the shell bands, including the aliphatic C–H pair, came from the samples and not from airborne fibres or handling.

Sample preparation

Each shell was washed with ultrapure Milli-Q water (18.2 M Ω ·cm) to remove soil and loose debris. The inner shell membrane was peeled off with stainless-steel forceps, because it holds organic material of its own that would otherwise interfere with the infrared reading (Hincke et al., 2012). The shells were rinsed again, air-dried under cover in a low-dust area, then oven-dried at 50–60°C for 24 h to drive off remaining moisture without harming the carbonate structure, and finally ground to a fine, even powder in an agate mortar and pestle, which avoids the plastic grinding media that could add contamination.

ATR-FTIR measurement

Spectra were recorded on an FTIR-7600 Fourier-transform infrared spectrometer (Lambda Scientific Systems, Inc., LSS) fitted with a diamond ATR accessory, over 4000–400 cm⁻¹ at 4 cm⁻¹ resolution, averaging 32 scans, with a background recorded and subtracted before each measurement. Spectra were processed and peaks picked in the instrument's Spectrum Analysis software. A deuterated triglycine sulphate (DTGS) detector was used. A single spectrum was recorded per shell, and no ATR penetration-depth correction was applied.

Data handling and statistics

Bands were assigned by comparing their positions with published values for calcite and for polymer functional groups (Hincke et al., 2012; Rodríguez-Navarro et al., 2015; Harrison et al., 2012; Gewert et al., 2015). Because absorbance is roughly proportional to how much of a component is present, the two aliphatic C–H bands (~ 2927 and ~ 2869 cm⁻¹) were divided by the carbonate reference band (~ 1423 cm⁻¹) to give dimensionless ratios that correct for differences in overall signal, such as how well each powder sat on the crystal. For each site the detection frequency of the C–H bands and the mean $\pm$ standard deviation (SD) of each ratio was computed, with the coefficient of variation (CV) as a measure of spread. To test whether the average ratio differed between sites, the two-sided Mann–Whitney U test was used, with the rank-biserial correlation as an effect size; to test whether the spread differed, Levene's test (median-centred) was used and confirmed with the Fligner–Killeen test. Significance was set at $p < 0.05$. With seven and eight shells, the study is small and clearly preliminary, and single measurements were made per shell; the statistics describe the data honestly rather than settle the question. Analyses were run in Python 3 using SciPy. No large language model was used to generate, analyse, or interpret data; Claude (Anthropic) was used only for language editing of author-written text.

Results and discussion

Feasibility of eggshell ATR-FTIR and the calcite framework

Every one of the 15 spectra was dominated by calcium carbonate, and the pattern was so consistent that the shells could almost have been laid over one another (Figs. 3–6; band positions in Table 1). The strongest feature in each spectrum, near 1423 cm⁻¹, is the carbonate ν_3 asymmetric C–O stretch, the

signature band of calcite and by far the largest absorbance in the scan. It never moved by more than a few wavenumbers from shell to shell, and its absorbance was high and steady, averaging 0.977 ± 0.091 AU at Rau and 0.950 ± 0.032 AU at Jamli, showing that the two sites produced shells of essentially the same mineral make-up. This band was accompanied in every shell by the ν_2 out-of-plane bend near 874 cm^{-1} , the ν_4 in-plane bend near 710 cm^{-1} , and the calcite overtone bands near 2516 and 1797 cm^{-1} . Such a complete, well-ordered calcite pattern in all 15 shells shows that the mineral framework was intact, with no broadening or extra bands to suggest weathering or dissolution, and that it forms a dependable internal standard against which weaker organic bands can be read (Hincke et al., 2012; Rodríguez-Navarro et al., 2015). On this evidence alone the first aim is met: ATR-FTIR describes lapwing eggshells reliably and repeatably, on material that costs nothing and harms no bird, and the steadiness of the reference band is what makes the ratios below trustworthy. This is the study’s practical contribution and it stands regardless of what the organic signal means.

Table 1

Characteristic ATR-FTIR band positions (cm^{-1}) observed across all 15 Red-wattled Lapwing eggshells, with assignments. Positions were consistent across shells and sites; representative ranges are given. The band near 1797 cm^{-1} is the calcite $2\nu_2$ overtone, not a carbonyl

Band range (cm^{-1})	Assignment	Origin
~ 3389–3450	O–H / N–H stretch	Adsorbed water / organic matrix
~ 2916–2947	Aliphatic C–H asymmetric stretch	Organic (non-specific)
~ 2854–2885	Aliphatic C–H symmetric stretch	Organic (non-specific)
~ 2488–2541	Carbonate overtone / combination	Calcite
~ 1779–1822	Calcite $2\nu_2$ overtone (not carbonyl)	Calcite
~ 1395–1441	Carbonate ν_3 asymmetric C–O stretch	Calcite (reference band)
~ 856–888	Carbonate ν_2 out-of-plane bend	Calcite
~ 698–725	Carbonate ν_4 in-plane bend	Calcite
~ 615–652	Weak mineral / lattice mode	Mineral
~ 514–546	Weak mineral / lattice mode	Mineral
~ 423–481	Mineral lattice mode	Mineral

A weak but consistent organic signal, honestly interpreted

Sitting on top of the carbonate background, every shell also showed a weak pair of aliphatic C–H bands, near 2927 cm^{-1} (asymmetric $-\text{CH}_2/-\text{CH}_3$ stretch) and 2869 cm^{-1} (symmetric stretch), detected in all 7 Rau shells and all 8 Jamli shells (100%). The blank was essentially empty here (0.003 and 0.002 AU), one to two orders of magnitude below the shell values ($0.147\text{--}0.321$ AU at 2927 cm^{-1}), so the signal is real and not laboratory or airborne contamination. What that signal is made of, however, is a separate matter.

Aliphatic C–H groups occur in synthetic polymers such as polyethylene and polypropylene, but they are equally the backbone of the proteins and lipids of the eggshell itself; membrane removal and rinsing reduce the shell's own contribution, but no infrared band at 2927 cm^{-1} can say whether its carbon chain is synthetic or biological (Harrison et al., 2012; Weisser et al., 2021). The bands are therefore reported as consistent with, but not proof of, plastic-related material.

Two features of the spectra guard against over-reading. The band near 1797 cm^{-1} , which might be mistaken for a weathering carbonyl, sits at almost exactly twice the 874 cm^{-1} band, holds a fixed position across shells, and rises and falls with the other carbonate bands; it is the calcite $2\nu_2$ overtone, not a carbonyl. A genuine oxidised-polymer carbonyl would appear nearer $1715\text{--}1740\text{ cm}^{-1}$ (Gewert et al., 2015), and that region stayed flat. Likewise, the 710 and 874 cm^{-1} bands are carbonate ν_4 and ν_2 modes, not aromatic ring vibrations. We flag the 1797 cm^{-1} overtone in particular because, in a calcite-rich matrix, mistaking it for a carbonyl would manufacture support for plastic that the spectra do not provide. The organic case thus rests on the C–H doublet alone.

Comparison between sites: same average, different spread

Normalised to the carbonate band, the two sites were alike on average. The $2927/1423$ ratio was 0.247 ± 0.094 at Rau and 0.222 ± 0.020 at Jamli, and the $2869/1423$ ratio was 0.188 ± 0.030 at Rau and 0.168 ± 0.022 at Jamli (Table 2). Neither difference approached significance (Mann–Whitney: $U = 28$, $p = 1.00$, rank-biserial $r = 0.00$ for $2927/1423$; $U = 40$, $p = 0.19$, $r = -0.43$ for $2869/1423$). A peri-urban town and a semi-rural village about 15 km apart therefore carried a comparable weak organic signal on their eggshells, and no urban-versus-rural gradient in the means is claimed.

Table 2

Aliphatic C–H / carbonate absorbance ratios (normalised to the $\sim 1423\text{ cm}^{-1}$ calcite ν_3 band) for each of the 15 eggshells, with site means $\pm$ SD and coefficient of variation (CV). Means did not differ between sites (Mann–Whitney U); the spread of the 2927/1423 ratio was significantly greater at Rau (Levene $p = 0.005$)

Sample	2927 AU	2869 AU	2927/1423 ; 2869/1423
Rau-1	0.214	0.167	0.223 ; 0.174
Rau-2	0.307	0.181	0.345 ; 0.204
Rau-3	0.169	0.226	0.152 ; 0.203
Rau-4	0.284	0.143	0.303 ; 0.153
Rau-5	0.196	0.209	0.186 ; 0.199
Rau-6	0.321	0.128	0.372 ; 0.148
Rau-7	0.147	0.238	0.143 ; 0.232
Rau mean $\pm$ SD	0.234 $\pm$ 0.070	0.185 $\pm$ 0.042	0.247 $\pm$ 0.094 ; 0.188 $\pm$ 0.030
Rau CV	-	-	38.0% ; 16.2%
Jamli-1	0.206	0.159	0.218 ; 0.168
Jamli-2	0.237	0.147	0.242 ; 0.150
Jamli-3	0.184	0.176	0.201 ; 0.192
Jamli-4	0.221	0.138	0.231 ; 0.144
Jamli-5	0.195	0.169	0.209 ; 0.181
Jamli-6	0.246	0.154	0.246 ; 0.154
Jamli-7	0.173	0.183	0.191 ; 0.202
Jamli-8	0.228	0.144	0.235 ; 0.149
Jamli mean $\pm$ SD	0.211 $\pm$ 0.026	0.159 $\pm$ 0.016	0.222 $\pm$ 0.020 ; 0.168 $\pm$ 0.022
Jamli CV	-	-	9.0% ; 13.1%
Procedural blank	0.003	0.002	1423 blank = 0.004 AU

The clear difference lay in the spread. At Rau the 2927/1423 ratio ranged from 0.143 to 0.372, the widest in the dataset, with a coefficient of variation of 38.0%; at Jamli it stayed between 0.191 and 0.246, with a CV of only 9.0%. This difference in spread was significant on two tests (Levene $p = 0.005$; Fligner–Killeen $p = 0.015$). A difference in variance rather than mean has a sensible reading: at a growing peri-urban site, contamination tends to be patchy, with waste dumped and dispersed unevenly so that some nests pick up more surface material than others, whereas a rural village presents a more uniform background. This

is the strongest and most defensible result in the study, but with seven and eight shells it is a lead to be tested, not a settled mechanism.

Placed alongside the microscopy, which showed suspected microplastic debris - fibres, films, fragments and tyre-wear particles - in the soil and dust at both sites, and the informal dumping nearby (with Jamli flanked by an uncontrolled dump), the weak shell signal is enough to justify the study's central suggestion: that these sites and samples deserve confirmatory, polymer-specific analysis. It is not enough to claim that plastic has been detected on the shells, and the convergence of circumstantial lines is deliberately not allowed to harden into a claim the spectra cannot support. That a rural village carried the same eggshell signal as a peri-urban town also fits the wider view that plastic-related pollution on land is diffuse, carried by agricultural plastics, informal waste disposal and atmospheric deposition rather than confined to city centres (Allen et al., 2019; Mahreen et al., 2025; Prajapati et al., 2025).

Possible routes of exposure

Because egg contents and adult birds were not analysed, this study cannot say whether organic material was built into the shell by the mother or picked up on its surface after laying. Both are plausible and not mutually exclusive: maternal transfer of metals and persistent pollutants into eggs is well documented in birds and could in principle extend to plastic-associated chemicals (Eagles-Smith et al., 2016), while a scrape nest on bare ground keeps the shell pressed against soil and coated in settling dust throughout incubation, so surface pick-up by the porous calcite-protein shell is at least as likely (Hincke et al., 2012). For now the eggshell signal is best read as a rough, integrated index of environmental exposure rather than a readout of any single route; separating the routes would need paired analysis of shells, egg contents, nest substrate and adults.

Limitations and next steps

The limitations are stated plainly: the sample is small (seven and eight shells); each shell was measured once; the eggshells were characterised with a single technique that cannot identify a polymer; and the soil and dust were examined only by microscopy, which flags suspected microplastics by shape but confirms nothing. No claim is therefore made about which polymer is present, how much, or whether the shell signal and the soil particles share an origin. The path forward follows from these limits. More shells across more nests and sites would give the variance difference real statistical power. Confirmatory chemistry - micro-Raman spectroscopy and pyrolysis-GC/MS on both the shells and the environmental particles - would allow direct matching and turn today's circumstantial suggestion into a firm finding or a clean negative (Käppler et al., 2018). Widening the sampling to egg contents, adult tissues and nest material would separate maternal from external routes, and element-specific methods (ICP-MS, EDX, XRF) would be needed for any metal question, since low-frequency FTIR bands cannot identify a metal. With these additions, eggshell ATR-FTIR could grow from the feasibility demonstration presented here into a validated part of the toolkit for monitoring plastic-related pollution in ground-nesting birds.

Conclusions

ATR-FTIR can be applied to non-invasively collected Red-wattled Lapwing eggshells to describe their calcite framework and to detect a weak, consistent aliphatic C–H organic signal, making the eggshell a practical, rapid, non-destructive matrix for environmental chemical screening. Set against a habitat that visibly holds suspected microplastic debris and lies close to informal garbage dumping, this signal is a preliminary suggestion of plastic-related contamination, offered as a lead for confirmatory work rather than a detection of plastic. The signal was comparable at the peri-urban and semi-rural sites but significantly more variable at the peri-urban site, hinting at patchier exposure. No carbonyl or aromatic band was found, and the 1797 cm^{-1} band is the calcite overtone, an interpretive pitfall flagged here for future eggshell studies. Confirmation now depends on polymer-specific methods, matched environmental sampling and larger samples.

Declarations

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Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: This is an observational, non-invasive study based solely on naturally hatched and abandoned eggshell fragments of a common, non-protected bird species. No live birds, adults, active nests or viable eggs were handled or disturbed. No procedure was performed on any living animal, and therefore the study did not require animal-experimentation ethics approval.

Author contributions All authors contributed to the conception and design of the study. Sample collection, laboratory analyses, and data collection were performed by K.P. Data analysis and interpretation were carried out by K.P. and H.V. The first draft of the manuscript was written by K.P., and V.K.S. reviewed the final draft. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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Statement on Ethical Responsibilities of Authors All authors have read, understood, and complied, as applicable, with the journal's statement on the Ethical Responsibilities of Authors provided in the Instructions for Authors.

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Figures



1a



Fig. 1b

Figure 1

a Satellite view of the Rau study site (peri-urban), Indore district; the pin marks the sampling area and the outline the built-up extent. Scale bar 1 km. Source Google Earth

b Satellite view of the Jamli study site (semi-rural village), Dr. Ambedkar Nagar (Mhow) Block, Indore district; the pin marks the village and the outline its extent, with farmland and a waterbody around it.

Scale bar 0.5 km. Source Google Earth

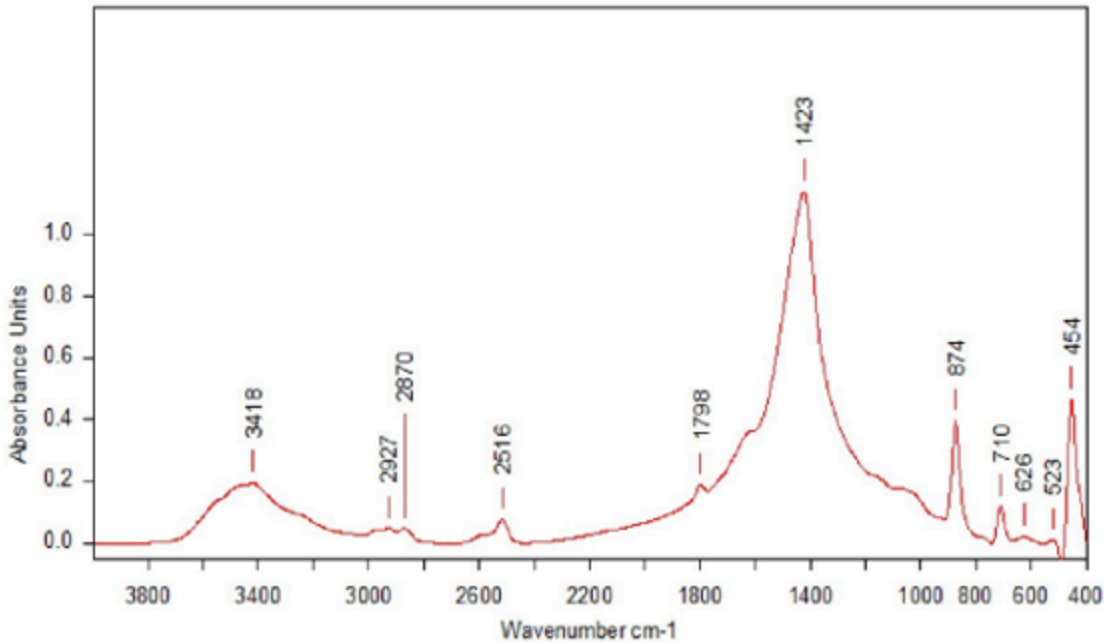


Figure 2

Representative non-invasively collected Red-wattled Lapwing eggshell fragments in glass petri dishes before cleaning, showing the speckled shell and adhering soil and dust; only naturally hatched or abandoned shells were collected, and no live birds, active nests or viable eggs were disturbed



Figure 3

Representative ATR-FTIR absorbance spectrum of a Rau eggshell (4000–400 cm^{-1}), dominated by calcite bands (~ 1423 , 874, 710 cm^{-1} ; overtones ~ 2516 and ~ 1797 cm^{-1}) with weak aliphatic C–H bands near 2927 and 2869 cm^{-1}

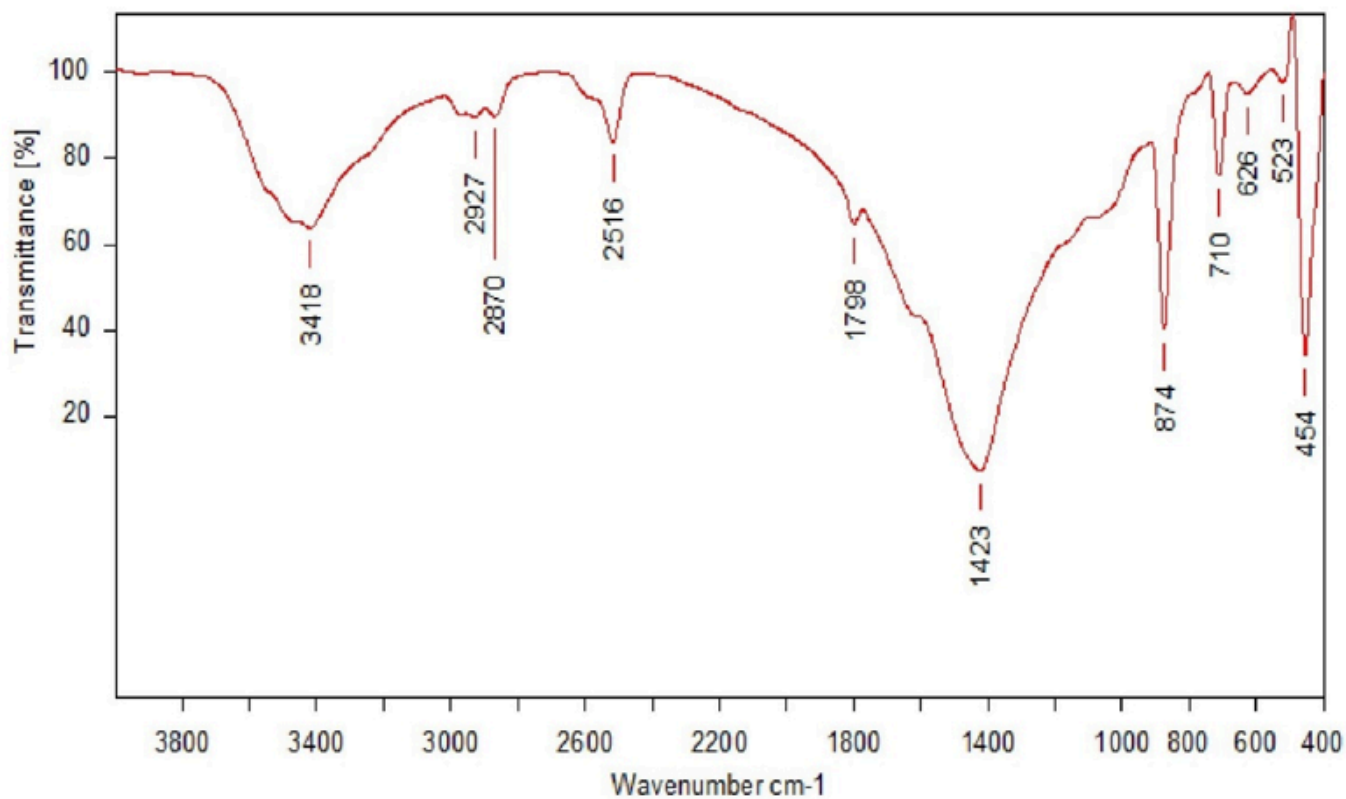


Figure 4

The same Rau eggshell shown as transmittance; all quantification used absorbance

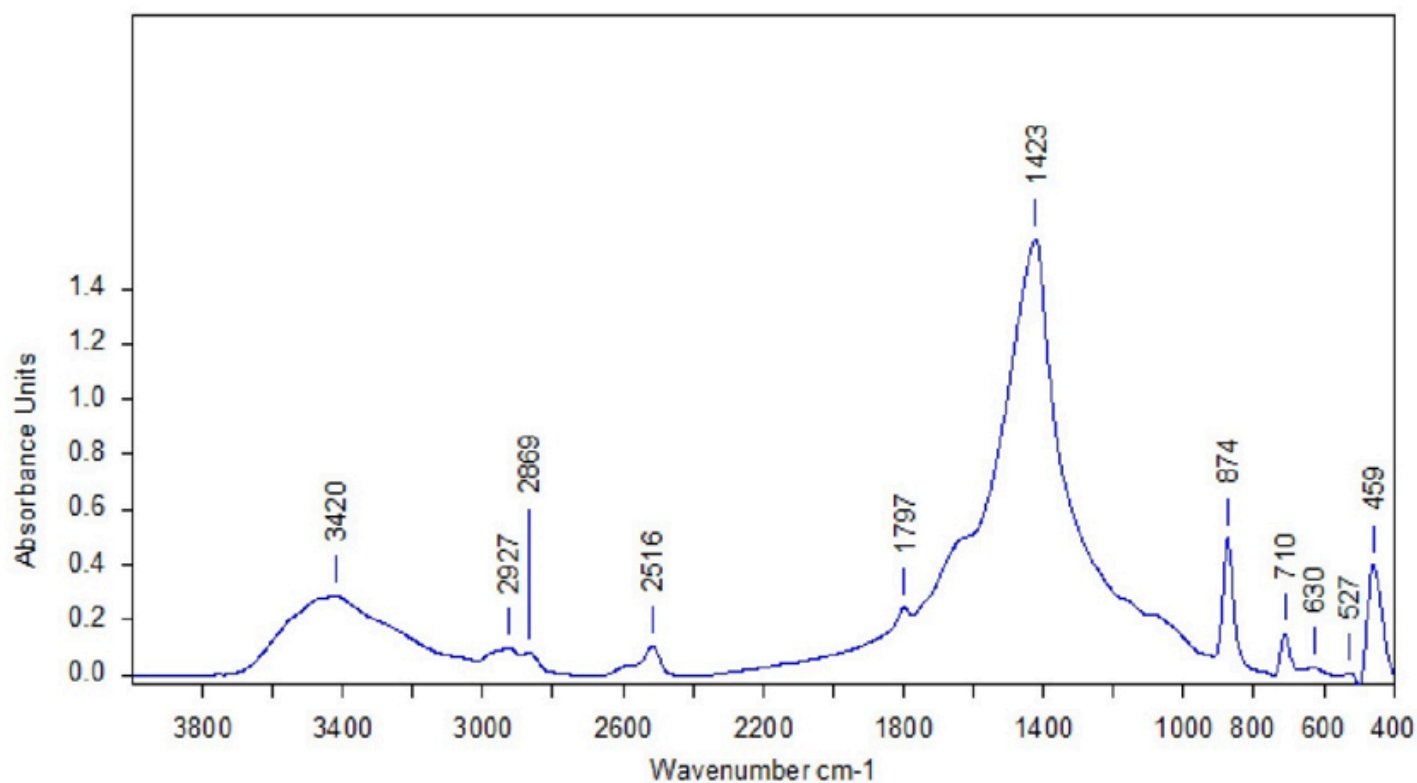


Figure 5

Representative ATR-FTIR absorbance spectrum of a Jamli eggshell; band positions match the Rau shell

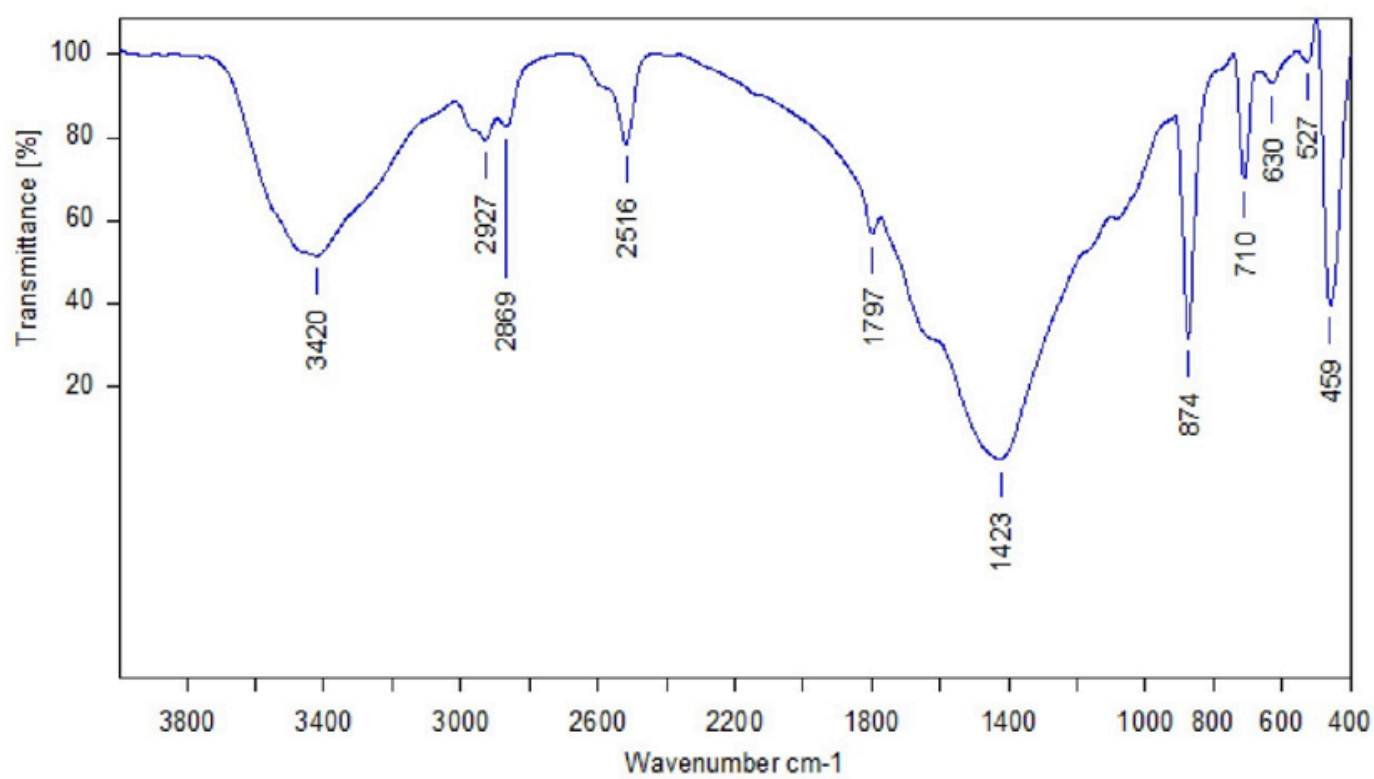


Figure 6

The same Jamli eggshell shown as transmittance

